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Development of a novel real-time RT-PCR assay for the dual detection of canine and phocine distemper virus.



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Objective:

As part of an overall goal to replace conventional PCR assays in the PCR diagnostics unit at DTU National Veterinary Institute with real-time PCR assays, we have developed a real-time RT-PCR assay for the detection of both canine and phocine distemper virus.

Conclusion:

- The novel real-time RT-PCR assay has a broad dynamic range and an efficiency of ~100%.
- Compared to an in-use RT-PCR assay [1] the real-time RT-PCR has a higher sensitivity and specifically detects canine and phocine distemper virus.

Introduction

Canine and phocine distemper virus both belong to the genus *Morbillivirus* within the virus family *Paramyxoviridae*, and have a negative-sense, single-stranded RNA genome. Canine distemper virus is known to infect a wide range of species belonging to *Canidae* and *Mustelidae* whereas phocine distemper virus is found in *Pinnipedia*. Both viruses can cause high morbidity and mortality.

Materials & methods

A set of primers and a dual labelled probe was designed based on an alignment, using CLC Main Workbench software, of distemper sequences in GenBank from various species and in-house sequences from recent outbreaks in Danish farmed mink. The assay amplifies a segment of 151 bp in the Phosphoprotein (P) gene of the distemper virus genome. Experimental data was obtained using the AgPath-ID™ One-Step RT-PCR kit (Applied Biosystems™) and a RotorGene Q real-time PCR machine (QIAGEN). Dynamic range and PCR efficiency (E) was experimentally determined using 10-fold dilutions of a specifically designed distemper DNA-oligo and extracted RNA from positive clinical samples. The sensitivity of the real-time RT-PCR assay was compared to the RT-PCR assay [1] already employed by the department.

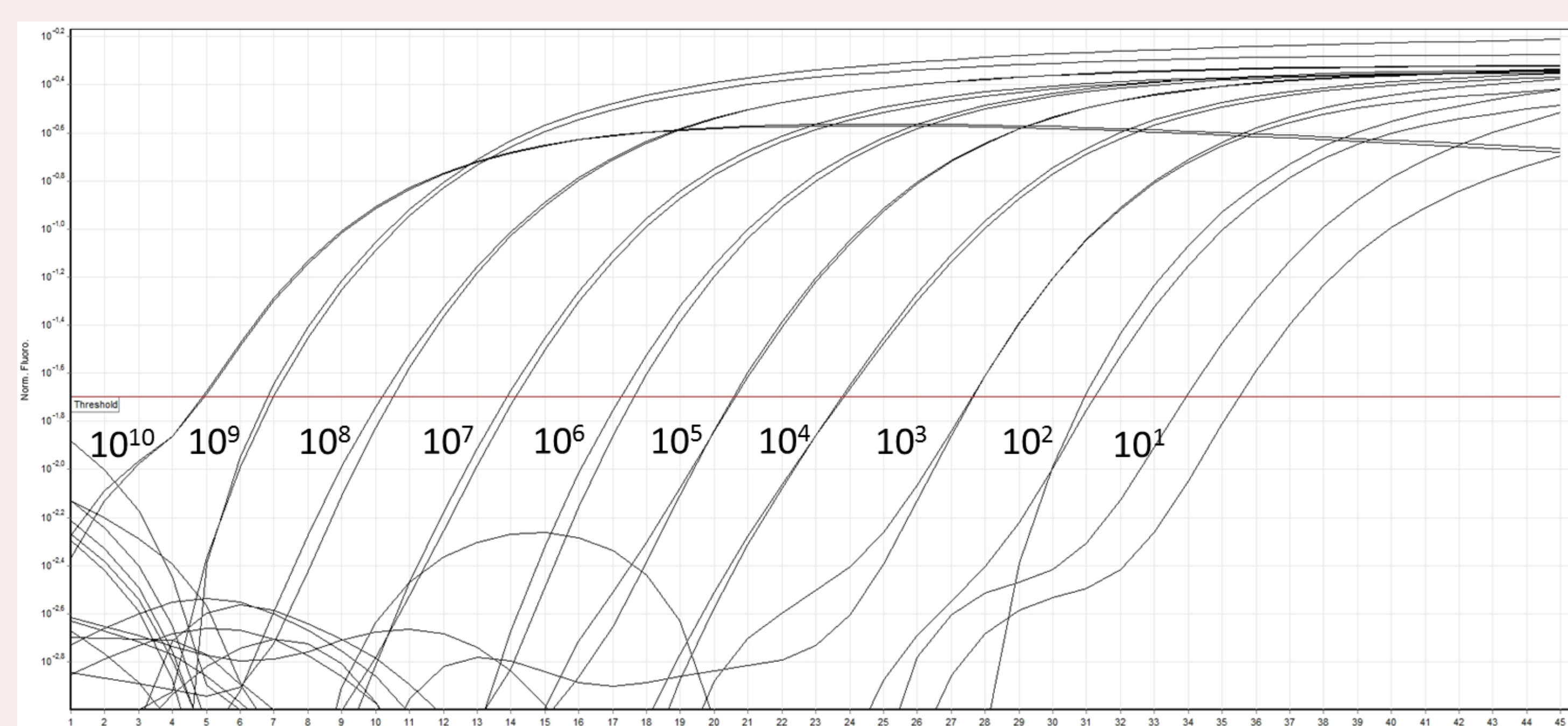


Figure 1: Experimental data from a real-time RT-PCR setup using a dilution series of a specially designed distemper DNA-oligo as template.

The copy number indicated by each duplicate curves is based on a concentration measurement followed by a copy number calculation, and refers to the total number of DNA copies in a given reaction.

Results

The dynamic range of the newly developed real-time RT-PCR assay was experimentally shown to be 9 log values for the DNA-oligo (Figure 1), and 7 log values for clinical samples. The PCR efficiency was calculated to range between 0.95 and 1.05 with a high degree of repeatability, both when using a dilution series of the distemper DNA-oligo and of clinical samples.

Comparison between the real-time RT-PCR assay and the currently used assay, showed that the real-time assay detects a 10^2 –fold further dilution of clinical sample with a higher degree of certainty (Figure 2). Furthermore, testing of distemper positive clinical samples from a wide range of different species performed in parallel with the RT-PCR assay, revealed that whereas both assays detect canine and phocine distemper virus, only the conventional assay could detect the dolphin distemper variant.

Future perspectives

We plan to study the PCR inhibition further using spiking experiments. In addition, tests will be performed on an elaborate panel of potential differential diagnostic agents in order to establish assay specificity.

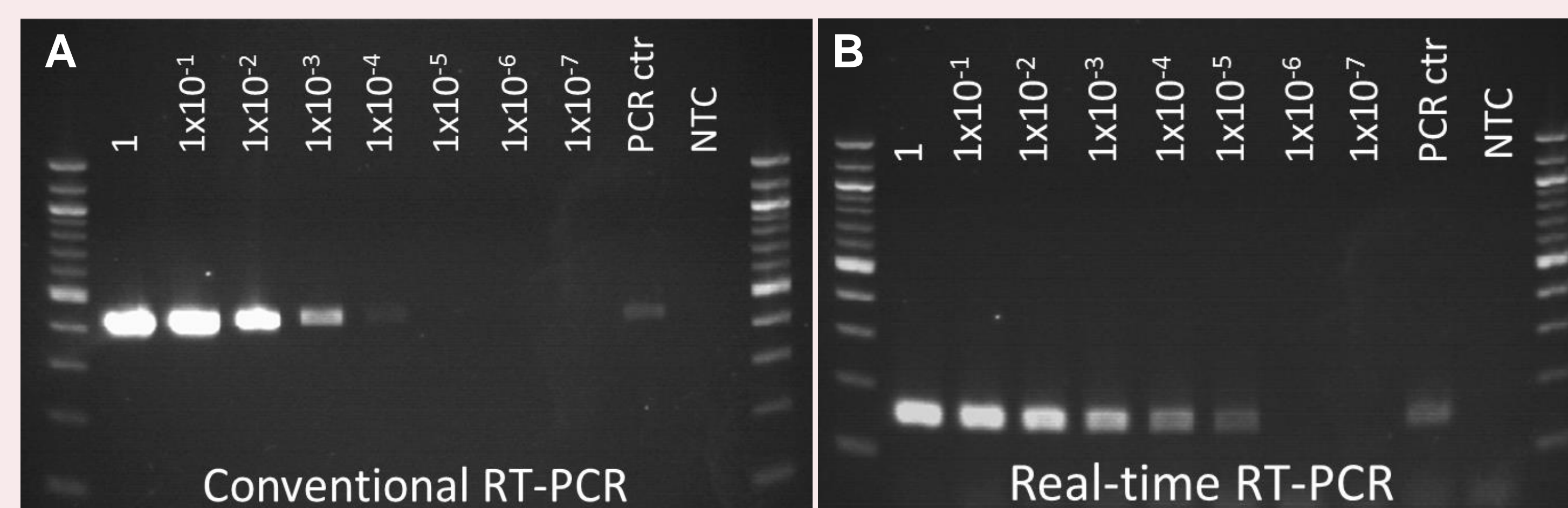


Figure 2: Comparison of the assay sensitivity between the distemper RT-PCR [1] and our novel real-time RT-PCR.

PCR on a 10-fold dilution series was performed with the RT-PCR assay [1] (A) and with the novel real-time RT-PCR assay (B). Purified RNA used for the dilution series originates from clinical mink material. PCR products were analyzed using 2% agarose E-gels (Invitrogen).

References:

1. Barrett, T. et al. "Dolphin and Porpoise Morbilliviruses Are Genetically Distinct from Phocine Distemper Virus." *Virology* 193.2 (1993): 1010–1012. Web. 12 Oct. 2016.